

Renumbered Claims

Application No. 09 811,842
Filed: March 19, 2001

Docket No. 330012-00002

Amendments

Please amend claims as follows:

Claims 1-44 (cancelled).

45 (cancelled).

46 (cancelled).

47 (cancelled).

48 (cancelled).

49 (cancelled).

50 (cancelled).

51-52 (cancelled).

~~53~~ (previously added). A method for elucidating a protein expression profile of a test cell line or group of cells, the method comprising:

randomly introducing into the genome of a cell or group of cells a promoterless polynucleotide construct, the construct comprising in a 5' to 3' orientation:

- i) a splice acceptor consensus sequence;
- ii) the complementary sequence of a type IIS restriction enzyme recognition sequence;
- iii) an oligonucleotide sequence encoding an assayable marker peptide;
- iv) a polyadenylation sequence;

wherein said promoterless polynucleotide construct when introduced into an actively expressed genes results in the generation of truncated cellular protein fused at its C-terminal to the marker peptide;

- v) identifying those cells expressing said marker peptide fused to said truncated cellular protein;

- vi) determining the identity of the truncated proteins to which the marker peptide is fused in each group of sorted cells.

²
~~54~~ (currently amended). The method of claim ¹~~54~~ further comprising sorting cells identified in step v) into ~~monoclonal or polyclonal subgroups~~ subpopulations based on their different levels of expression of said marker peptide.

³
~~55~~ (currently amended). The method of claim ¹~~55~~, wherein the identity of the protein to which the marker peptide is fused is determined using a method selected from the group consisting of 5' Rapid Amplification of cDNA Ends (5' RACE) and Serial Analysis of Viral Integration (SAVI).

⁴
~~56~~ (currently amended). The method of claim ³~~56~~ wherein Serial Analysis of Viral Integration (SAVI) is performed by:

- i) isolating mRNA from each subgroup of cells;
- ii) reverse transcribing the mRNA into double stranded cDNA;
- iii) subjecting the cDNA to a restriction enzyme that recognizes the type IIS restriction enzyme recognition sequence, and cleaves the cDNA upstream of the recognition sequence, thereby generating one or more cDNA fragments, wherein each of these fragments comprise the oligonucleotide sequence corresponding to an upstream exon directly fused to the marker peptide, the type IIS restriction enzyme recognition sequence and a portion of a native sequence corresponding to the peptide marker;
- iv) adding an adaptor sequence to the end of the unknown oligonucleotide sequence;
- v) amplifying by the polymerase chain reaction, the fragments containing the oligonucleotide sequences of the exons fused to the marker peptide with oligonucleotide primers complementary to the adaptor and peptide marker encoding sequences;
- vi) cloning and sequencing said amplified fragments; and
- vii) comparing the sequence of each oligonucleotide against oligonucleotide sequences in a one or more nucleotide sequence database thereby identifying one or more fusion proteins present in each subgroup of cells.

⁵
~~57~~ (currently amended). A method to identify differentially expressed proteins in two different populations of cells, the method comprising:

randomly introducing into the genomes of a reference group of cells and into the genomes of a test group of cells a promoterless polynucleotide construct, wherein the construct comprises, in a 5' to 3' orientation:

- i) a splice acceptor consensus sequence;
- ii) the complementary sequence of a type IIS restriction enzyme recognition sequence;
- iii) an oligonucleotide sequence encoding an assayable marker peptide;
- iv) a polyadenylation sequence;

thereby generating a population of randomly truncated cellular proteins fused at their C-terminal truncated end to the marker peptide

- v) sorting both groups of cells into ~~several monoclonal or polyclonal subgroups~~ subpopulations of cells based on their differential expression levels of the marker peptide;
- vii) determining the identity of the fusion proteins generated in each subgroup of sorted cells by following one of the following procedures; and
- viii) comparing by statistical methods the protein expression profiles obtained for the test group of cells against the protein expression profiles obtained for the reference group of cells, thereby identifying differences in the expression levels of fusion proteins among the two groups of cells.

⁶
~~58~~ (currently amended). The method of claim ~~60~~ ⁶~~57~~ wherein the identity of the protein to which the marker peptide is fused is determined by 5' RACE or SAVI.

¹⁰
~~59~~ (currently amended). The methods of claims ~~53-57~~ ^{1 5 6} or ~~58~~ where the peptide marker encoding sequence lacks a translation initiation codon and possesses a translation STOP codon.

¹¹
~~60~~ (currently amended). The methods of claims ~~53-57~~ ^{1 5 6} or ~~58~~ where the peptide marker encoding sequence lacks a translation initiation and STOP codons.

⁷
~~61~~ (previously added). The method of claim ~~56~~ ^{4 6} or ~~58~~ wherein addition of the adaptor sequence is performed by ligation of a double stranded adaptor.

⁸
~~62~~ (previously added). The method of claim ~~56~~ ^{4 6} or ~~58~~ wherein addition of the adaptor sequence is performed by poly-deoxyribonucleotide tailing extension.

¹²
~~63~~ (currently amended). The methods of claim ~~53-57~~^{1 5 4} or ~~58~~ wherein said separation of cells into subgroups subpopulations of cells based on the levels of expression of the peptide marker is performed by fluorescent activated cell sorting.

¹⁷
~~64~~ (currently amended). The methods of claims ~~53-57~~^{1 5 4} or ~~58~~ wherein the oligonucleotide sequence is a fluorescent protein coding oligonucleotide sequence.

¹⁸
~~65~~ (currently amended). The methods of claim ~~64~~¹⁷ wherein the fluorescent protein encoding oligonucleotide is a green fluorescent protein (GFP) coding sequence.

¹⁹
~~66~~ (currently amended). The method of claim ~~65~~¹⁸ wherein the GFP oligonucleotide coding sequence is a humanized retina renilla GFP (hrGFP) coding sequence.

¹³
~~67~~ (currently amended). The methods of claims ~~53-57~~^{1 5 6} or ~~58~~ wherein the protein coding sequence is an epitope recognized by fluorescently or enzymatically labeled antibodies.

¹⁴
~~68~~ (currently amended). The methods of claims ~~53-57~~^{1 5 4} or ~~58~~ wherein the marker peptide encoded by the polynucleotide requires interaction with another protein in order to generate a fluorescent signal.

²⁰
~~69~~ (currently amended). The methods of claims ~~53-57~~^{1 5 4} or ~~58~~ wherein the polynucleotide construct is introduced into the genome of the cell via a vector.

²¹
~~70~~ (currently amended). The methods of claim ~~69~~²⁰ wherein the vector is a viral vector.

²²
~~71~~ (currently amended). The methods of claim ~~70~~²¹ wherein the viral vector is selected from the group consisting of a retroviral vector, a lentiviral vector, an adenoviral vector, and an adeno-associated viral vector.

⁹
~~72~~ (currently amended). The methods of claims ~~53-57~~^{3,4 6} or ~~58~~ wherein following amplification of the one or more extended cDNA fragments, and prior to cloning and sequencing the one or more cDNA fragments, the fragments are ligated together to form a concatenated molecule.

¹⁵
~~73~~ (currently amended). The methods of claims ~~53-57~~^{1 5 6} or ~~58~~ wherein the polynucleotide construct further comprises, downstream of the oligonucleotide encoding a marker peptide and before the polyadenylation signal, an internal ribosome entry site followed by another protein expression marker.

¹⁶
~~74~~ (currently amended). The methods of claims ~~53-57~~^{1 5 6} or ~~58~~ wherein the polynucleotide construct further comprises, downstream of the oligonucleotide having a specified sequence, a sequence encoding, upon expression, a selectable marker.